

APPENDIX B

CLAIMS PENDING WITH ENTRY OF AMENDMENT

1. (Once Amended) A method of detecting chitinous material in a processed non-chitinous biological sample, said method comprising
contacting said biological sample with a probe that is a lectin that binds chitin;
contacting said biological sample with a pectinase;
filtering said sample;
washing said filter to remove unbound lectin;
eluting bound lectin with a chitin, a chitin degradation product or a chitin analogue; and
detecting said lectin wherein detection of said lectin indicates the presence of chitin in said biological sample.
2. The method of claim 1, wherein said chitin comprises an insect, an insect part, or any animal of the phylum Arthropoda subphylum Crustacea.
3. The method of claim 1, wherein said chitin is a component of a micro-organism.
4. The method of claim 3, wherein said microorganism is selected from the group consisting of a fungus, a mold, and a yeast.
5. The method of claim 3, wherein said microorganism is a selected from the group consisting of a fungus of phylum Ascomycota, a fungus of phylum Basidiomycota, a fungus of phylum Chytridiomycota, a fungus of phylum Zygomycota, and a member of the phylum Oomycota in the Stramenopila kingdom.
6. The method of claim 3, wherein said microorganism is a fungus selected from the group consisting of Cladosporium spp, Fusarium spp, Stemphylium spp, Alternaria spp, Geotrichum spp, Fusarium spp, Rhizopus spp, Botrytis spp, Phytophthora spp, Pythium spp, and Pythium spp.

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7. The method of claim 1, wherein said biological sample is a selected from the group consisting of an agricultural product, a food product, a wood product, a textile, and an animal tissue product.

8. The method of claim 7, wherein said agricultural product is selected from the group consisting of a fruit, a vegetable, a grain, forage, a silage, a juice, a wood, a flower, and a seed.

9. The method of claim 7, wherein said agricultural product is a fruit selected from the group consisting of a tomato, a pepper, a grape, an apple, an orange, a lemon, a berry.

10. The method of claim 1, wherein said lectin is selected from the group consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato lectin, potato lectin barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a Vibrio lectin, and a hevein.

11. The method of claim 1, wherein said method further comprises contacting said sample with a blocking reagent.

12. The method of claim 11, method wherein said blocking reagent is serum albumin.

16. (Once Amended) The method of claim 1, wherein said chitin degradation product is N-acetyl D-glucosamine.

17. The method of claim 1, wherein said lectin is labeled with a detectable label.

18. The method of claim 17, wherein said label is selected from the group consisting of a radioactive label, a magnetic label, a colorimetric label, an enzymatic label, a fluorescent label, a metal, an antibody, a biotin, and an avidin or streptavidin.

19. The method of claim 17, wherein said label is a fluorescent label.

20. The method of claim 19, wherein said detecting comprises using a fluorometer to detect fluorescence of said label.

22. The method of claim 20, wherein said fluorometer uses a bandpass filter.

23. The method of claim 20, wherein said fluorometer is a surface-reading fluorometer.

24. The method of claim 1, wherein said method is performed at a pH greater than about pH 7.

25. The method of claim 1, wherein said method is performed at about pH 8.

26. The method of claim 1, wherein said pectinase comprises an enzyme selected from the group consisting of polygalacturonases, pectinesterases, pectin lyases, and hemicellulases.

27. The method of claim 1, wherein the processed biological sample is a sample that has been subjected to an operation selected from the group consisting of comminuting, homogenizing, heating, evaporation, lyophylization, filtering, concentrating, filtering, fermenting, freezing, and blanching.

28. The method of claim 1, wherein
the biological sample is selected from the group consisting of a fruit, a vegetable, a fruit juice, and a vegetable juice;

said lectin is a fluorescently labeled lectin selected from the group consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato lectin, potato lectin, barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a Vibrio lectin, and a hevein;

said pectinase is a pectinase selected from the group consisting of polygalacturonases, pectinesterases, pectin lyases and hemicellulases;

said sample is processed by comminuting, homogenizing, heating, evaporation, lyophylization, filtering, concentrating, filtering, fermenting, freezing, and blanching; and

said detecting comprises detecting a signal from the fluorescent label labeling said lectin.

29. (Once Amended) A method of detecting chitinous material in a non-chitinous biological sample, said method comprising
- in a solution at a pH ranging from about pH 7 to about pH 9 contacting said biological sample with a fluorescently labeled probe that is a lectin that binds chitin;
 - filtering said sample;
 - washing said filter to remove unbound lectin;
 - eluting bound lectin with a chitin, a chitin degradation product or a chitin analogue; and
 - detecting said lectin wherein detection of said lectin indicates the presence of chitin in said biological sample.
30. The method of claim 29, wherein said chitin comprises an insect or insect part.
31. The method of claim 29, wherein said chitin is a component of a micro-organism.
32. The method of claim 31, wherein said microorganism is selected from the group consisting of a fungus, a mold, and a yeast.
33. The method of claim 31, wherein said microorganism is a fungus selected from the group consisting of *Cladosporium herbarum*, *Fusarium oxysporum*, and *Stemphylium botryosum*, *Alternaria alternata*, *Geotrichum candidum*, *Fusarium oxysporum*, *Rhizopus stolonifer*, *Botrytis cinerea*, *Phytophthora parasitica*, *Pythium aphanidermatum*, *Pythium ultimum*.
34. The method of claim 29, wherein said biological sample is selected from the group consisting of an agricultural product, a food product, a wood product, a textile, and an animal tissue product.
35. The method of claim 34, wherein said agricultural product is selected from the group consisting of consisting of a fruit, a vegetable, a grain, forage, a silage, a juice, a wood, a flower, and a seed.

36. The method of claim 34, wherein said agricultural product is a fruit selected from the group consisting a fruit, a vegetable, a grain, forage, a silage, a juice, a wood, a flower, and a seed.

37. The method of claim 29, wherein said lectin is selected from the group consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato lectin, potato lectin barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a Vibrio lectin, and a hevein.

38. The method of claim 29, wherein said method further comprises contacting said sample with a blocking reagent.

39. The method of claim 38, method wherein said blocking reagent is serum albumin.

43. (Once Amended) The method of claim 29, wherein said chitin degradation product is N-acetyl D-glucosamine.

44. The method of claim 29, wherein said lectin is labeled with a detectable label.

45. The method of claim 17, wherein said label is selected from the group consisting of a radioactive label, a magnetic label, a colorimetric label, an enzymatic label, a fluorescent label, a metal, an antibody, a biotin, and an avidin or streptavidin.

46. The method of claim 17, wherein said label is a fluorescent label.

47. The method of claim 19, wherein said detecting comprises using a fluorometer to detect fluorescence of said label.

49. The method of claim 20, wherein said fluorometer is a surface-reading fluorometer.

50. The method of claim 29, wherein said method is performed at a basic pH greater than about pH 7.5.

51. The method of claim 29, wherein said method is performed at a basic pH about pH 8.0.

52. The method of claim 29, wherein

the biological sample is selected from the group consisting of a fruit, a vegetable, a fruit juice, and a vegetable juice;

said lectin is a fluorescently labeled lectin selected from the group consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato lectin, potato lectin, barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a Vibrio lectin, and a hevein; and

said detecting comprises detecting a signal from the fluorescent label labeling said lectin.

53. The method of claim 29, further comprising contacting said biological sample with a pectinase.

54. The method of claim 53, wherein said pectinase is selected from the group consisting of polygalacturonases, pectinesterases, pectin lyases and hemicellulases.